

Appl. No. : 09/668,788
Filed : September 22, 2000

AMENDMENTS TO THE CLAIMS

1. (Currently Amended) A process for the production of a glycosyl diacylglycerol, a sterolglycoside, a glycocerebroside, an alkyl- β -D-glycopyranoside, or a phosphoglycolipid glycolipids in transgenic cells and/or organisms using a processive lipid glycosyl transferase that successively transfers a hexose residues to a lipid acceptor, comprising:

transferring ~~of~~ a nucleic acid molecule that codes for a protein having the enzymatic activity of a processive diacylglycerol glycosyltransferase to ~~the-a cells or organisms~~; and

expressing the protein having the enzymatic activity of a processive diacylglycerol glycosyltransferase under suitable regulatory sequences in the cells ~~or the organisms~~, and to produce a glycosyl diacylglycerol, a sterolglycoside, a glycocerebroside, an alkyl- β -D-glycopyranoside, or a phosphoglycolipid.

~~recovering of the glycolipids synthesized by the enzymatic activity of a processive diacylglycerol glycosyltransferase from the cells or the organisms if desired.~~

2. (Previously Amended) The process according to claim 1, wherein the nucleic acid molecule codes for a protein having the enzymatic activity of a processive lipid glycosyltransferase from *Bacillus subtilis* or *Staphylococcus aureus*.

E6
3. (Currently Amended) The process according to claim 1, wherein the ~~transgenic cells are~~ cell is selected from the group consisting of a plant cell, a yeast cell, and a bacterial cells, and the organism is a plant.

4. (Cancelled) The process according to Claim 1, wherein the glycolipids are selected from the group consisting of glycosyl diacylglycerols, sterolglycosides, glycocerebrosides, phosphoglycolipids, and any combination thereof.

5. (Currently Amended) The process according to Claim 1, wherein the glycosyl diacylglycerol, the sterolglycoside, the glycocerebroside, the alkyl- β -D-glycopyranoside, or the phosphoglycolipid glycolipids are is selected from the group consisting of

monoglycosyldiacylglycerol,

diglycosyldiacylglycerol,

triglycosyl diacylglycerol,

tetraglycosyldiacylglycerol,

Appl. No. : 09/668,788
Filed : September 22, 2000

glycosyl ceramide,
diglycosyl ceramide,
steryl glycoside,
steryl diglycoside,
glycosyl phosphatidylglycerol, and
diglycosyl phosphatidylglycerol.

6. (Currently Amended) The process~~Process~~ according to Claim 1, wherein the glycosyl diacylglycerol, the sterolglycoside, the glycocerebroside, the alkyl- β -D-glycopyranoside, or the phosphoglycolipidglycolipids are~~is~~ selected from the group consisting of

monoglucosyldiacylglycerol,
diglucosyldiacylglycerol,
triglucosyldiacylglycerol,
tetraglucosyldiacylglycerol,
glucosyl ceramide,
diglucosyl ceramide,
steryl glucoside,
steryl diglucoside,
glucosyl phosphatidylglycerol, and
diglucosylphosphatidylglycerol.

7. (Cancelled) Use of a nucleic acid molecule coding for a protein having the biological activity of a processive diacylglycerol glycosyltransferase or of a proteins having the biological activity of a processive diacylglycerol glycosyltransferase for processive glycosylation, in particular for production of glycolipids.

8. (Cancelled) Use according to claim 7, wherein the nucleic acid molecule codes for a protein having the biological activity of a processive diacylglycerol glycosyltransferase from *Bacillus subtilis* or *Staphylococcus aureus*.

9. (Cancelled) Use according to claim 7 or 8, wherein the processive glycosylation, in particular the production of glycolipids, takes place *in vivo* or *in vitro*.

Appl. No. : **09/668,788**
Filed : **September 22, 2000**

10. (Cancelled) Use according to one of claims 7 to 9 for the production of glycosyldiacyl glycerols and/or phosphoglycolipids.

11. (Cancelled) Use according to any one of claims 7 to 10 for the production of monoglycosyldiacylglycerol,
diglycosyldiacylglycerol,
triglycosyl diacylglycerol,
tetraglycosyldiacylglycerol,
glycosyl ceramide,
diglycosyl ceramide,
steryl glycoside,
steryl diglycoside,
glycosyl phosphatidylglycerol, and/or
diglycosyl phosphatidylglycerol.

12. (Cancelled) Use according to any one of claims 7 to 11 for the production of monoglucosyldiacylglycerol,
diglucoxyldiacylglycerol,
triglucoxyldiacylglycerol,
tetraglucoxyldiacylglycerol,
glucosyl ceramide,
diglucoxyleramide,
steryl glucoside,
steryl diglucoxyde,
glucosyl phosphatidylglycerol, and/or
diglucoxyphosphatidylglycerol.

13. (Cancelled) A composition of matter comprising tetraglucoxyldiacylglycerol.

14. (Cancelled) A composition of matter comprising glucosylphosphatidylglycerol.

15. (Cancelled) A composition of matter comprising diglucoxyphosphatidylglycerol.

E6
Cont

Appl. No. : 09/668,788
Filed : September 22, 2000

16. (Cancelled) Method of maintaining a food composition in an emulsified state, comprising applying to the food composition a composition according to any one of claims 13 to 15.

17. (Cancelled) Method of cleaning, comprising applying a composition according to any one of claims 13 to 15.

18. (Currently Amended) A-The process according to Claim 1, wherein the lipid acceptor is a secondary lipid acceptor, and wherein the secondary lipid acceptor is selected from the group consisting of a monohexosyldiacylglycerolipid, a dihexosyldiacylglycerolipid, a trihexosyldiacylglycerolipid, a tetrahexosyldiacylglycerolipid, a glycocerebroside, a dihexosylcerebroside, a sterolglycoside, a steroldiglycoside and a phosphoglycolipid.

19. (Previously Added) The process according to Claim 1, wherein the nucleic acid molecule codes for a protein having the enzymatic activity of a processive lipid glycosyl transferase that successively transfers glucose to a lipid acceptor.

Eb
20. (Previously Added) The process according to Claim 1, wherein the lipid acceptor is a primary lipid acceptor, and wherein the primary lipid acceptor is diacylglycerol, sterol, phosphatidylglycerol or ceramide.

21. (Cancelled) The process according to Claim 1, wherein the glycolipids are glucosyl diacylglycerols, sterolglucosides, glucocerebrosides or phosphoglucolipids.

22. (Currently Amended) A process for the production of a glycosyl diacylglycerol, a sterolglycoside, a glycocerebroside, an alkyl-β-D-glycopyranoside, or a phosphoglycolipid glycolipid in a transgenic cell or an organism by the use of a processive lipid glycosyl transferase that successively transfers a hexose residue to a lipid acceptor, comprising the steps of:

- transferring a nucleic acid molecule that codes for a protein having the enzymatic activity of a processive lipid glycosyl transferase to ~~the-a cell or the organism~~, the protein having an amino acid sequence which is at least ~~60~~70% identical to the sequence selected from sequences in the group consisting of SEQ ID NO. 2 and SEQ ID NO. 4; and

Appl. No. : 09/668,788
Filed : September 22, 2000

- expressing the protein having the enzymatic activity of a processive lipid glycosyl transferase under suitable regulatory sequences in the cell ~~or the organism; and to produce~~ a glycosyl diacylglycerol, a sterolglycoside, a glycocerebroside, an alkyl- β -D-glycopyranoside, or a phosphoglycolipid.

~~recovering the glycolipid synthesized by the enzymatic activity of the processive lipid glycosyl transferase from the cell or the organism, if desired.~~

23. (Previously Added) The process according to claim 22, wherein the protein having the enzymatic activity of a processive lipid glycosyl transferase has an amino acid sequence which is more than 80% identical to the sequence selected from the sequences in the group consisting of SEQ ID NO. 2 and SEQ ID NO. 4.

24. (New) The process according to Claim 22, further comprising recovering the glycosyl diacylglycerol, the sterolglycoside, the glycocerebroside, the alkyl- β -D-glycopyranoside, or the phosphoglycolipid synthesized by the enzymatic activity of the processive lipid glycosyl transferase from the cell.

25. (New) The process according to claim 22, wherein the cell is selected from the group consisting of a plant cell, a yeast cell, and a bacterial cell.

26. (New) The process according to claim 22, wherein the protein having the enzymatic activity of a processive diacylglycerol glycosyltransferase comprises an amino acid sequence selected from the group consisting of MITKPGGITXTE (SEQ ID NO. 8), VKXTGIP (SEQ ID NO. 9), ZPDIIIIXXXP (SEQ ID NO. 10), more than 5 amino acids from within the sequence EHQPDIII (SEQ ID NO. 5), more than 6 amino acids from within the sequence QVVVVCGKN (SEQ ID NO. 6), and more than 6 amino acids from within the sequence DCMITKPG (SEQ ID NO. 7).

27. (New) A process for the production of a glycosyl diacylglycerol, a sterolglycoside, a glycocerebroside, an alkyl- β -D-glycopyranoside, or a phosphoglycolipid using a bacterial processive lipid glycosyl transferase that successively transfers a hexose residue to a lipid acceptor, comprising:

E6
CONT

transferring a nucleic acid molecule that codes for a protein having the enzymatic activity of a bacterial processive diacylglycerol glycosyltransferase to a cell; and

Appl. No. : 09/668,788
Filed : September 22, 2000

expressing the protein having the enzymatic activity of a bacterial processive diacylglycerol glycosyltransferase under suitable regulatory sequences in the cell to produce the glycosyl diacylglycerol, the sterolglycoside, the glycocerebroside, the alkyl- β -D-glycopyranoside, or the phosphoglycolipid.

28. (New) The process according to Claim 27, further comprising recovering the glycosyl diacylglycerol, the sterolglycoside, the glycocerebroside, the alkyl- β -D-glycopyranoside, or the phosphoglycolipid synthesized by the enzymatic activity of the bacterial processive diacylglycerol glycosyltransferase from the cell.

29. (New) The process according to Claim 27, wherein the glycosyl diacylglycerol, the sterolglycoside, the glycocerebroside, the alkyl- β -D-glycopyranoside, or the phosphoglycolipid is selected from the group consisting of:

monoglycosyldiacylglycerol,
diglycosyldiacylglycerol,
triglycosyl diacylglycerol,
tetraglycosyldiacylglycerol,
glycosyl ceramide,
diglycosyl ceramide,
steryl glycoside,
steryl diglycoside,
glycosyl phosphatidylglycerol, and
diglycosyl phosphatidylglycerol.

30. (New) The process according to Claim 27, wherein the glycosyl diacylglycerol, the sterolglycoside, the glycocerebroside, the alkyl- β -D-glycopyranoside, or the phosphoglycolipid is selected from the group consisting of:

monoglucosyldiacylglycerol,
diglucosyldiacylglycerol,
triglucosyldiacylglycerol,
tetraglucosyldiacylglycerol,
glucosyl ceramide,
diglucosyl ceramide,

Appl. No. : 09/668,788
Filed : September 22, 2000

steryl glucoside,
steryl diglucoside,
glucosyl phosphatidylglycerol, and
diglucosylphosphatidylglycerol.

31. (New) The process according to Claim 27, wherein the lipid acceptor is a secondary lipid acceptor, and wherein the secondary lipid acceptor is selected from the group consisting of a monohexosyldiacylglycerolipid, a dihexosyldiacylglycerolipid, a trihexosyldiacylglycerolipid, a tetrahexosyldiacylglycerolipid, a glycocerebroside, a dihexosylcerebroside, a sterolglycoside, a steroldiglycoside and a phosphoglycolipid.

32. (New) The process according to Claim 27, wherein the nucleic acid molecule codes for a protein having the enzymatic activity of a bacterial processive lipid glycosyl transferase that successively transfers glucose to a lipid acceptor.

33. (New) The process according to Claim 27, wherein the lipid acceptor is a primary lipid acceptor, and wherein the primary lipid acceptor is diacylglycerol, sterol, phosphatidylglycerol or ceramide.

34. (New) The process according to claim 27, wherein the cell is selected from the group consisting of a plant cell, a yeast cell, and a bacterial cell.

35. (New) The process according to claim 27, wherein the nucleic acid molecule codes for a protein having the enzymatic activity of a bacterial processive lipid glycosyltransferase from a *bacillus* or a *staphylococcus*.

36. (New) A process for the production of a glycosyl diacylglycerol, a sterolglycoside, a glycocerebroside, an alkyl- β -D-glycopyranoside, or a phosphoglycolipid in a cell by the use of a processive lipid glycosyl transferase that successively transfers a hexose residue to a lipid acceptor, comprising the steps of:

- transferring a nucleic acid molecule that codes for a protein having the enzymatic activity of a processive lipid glycosyl transferase to the cell, the protein having an amino acid sequence which is at least 90% identical to the sequence selected from sequences in the group consisting of SEQ ID NO. 2 and SEQ ID NO. 4; and

- expressing the protein having the enzymatic activity of a processive lipid glycosyl transferase under suitable regulatory sequences in the cell to produce a glycosyl

E
Cmt

Appl. No. : 09/668,788
Filed : September 22, 2000

diacylglycerol, a sterolglycoside, a glycocerebroside, an alkyl- β -D-glycopyranoside, or a phosphoglycolipid.

37. (New) The process according to Claim 36, further comprising recovering the glycosyl diacylglycerol, the sterolglycoside, the glycocerebroside, the alkyl- β -D-glycopyranoside, or the phosphoglycolipid synthesized by the enzymatic activity of the processive lipid glycosyl transferase from the cell.

38. (New) The process according to claim 36, wherein the protein having the enzymatic activity of a processive lipid glycosyl transferase has an amino acid sequence which is more than 95% identical to the sequence selected from the sequences in the group consisting of SEQ ID NO. 2 and SEQ ID NO. 4.

39. (New) The process according to Claim 36, wherein the protein having the enzymatic activity of a processive lipid glycosyl transferase has an amino acid sequence which is identical to the sequence selected from the sequences in the group consisting of SEQ ID NO. 2 and SEQ ID NO. 4.

40. (New) A process for the production of a glycosyl diacylglycerol, a sterolglycoside, a glycocerebroside, an alkyl- β -D-glycopyranoside, or a phosphoglycolipid by the use of a processive lipid glycosyl transferase that successively transfers a hexose residue to a lipid acceptor, comprising the steps of:

- obtaining a nucleic acid molecule that codes for a protein having the enzymatic activity of a processive lipid glycosyl transferase, the protein having an amino acid sequence which is at least 90% identical to the sequence selected from sequences in the group consisting of SEQ ID NO. 2 and SEQ ID NO. 4; and

- expressing the protein having the enzymatic activity of a processive lipid glycosyl transferase to produce a glycosyl diacylglycerol, a sterolglycoside, a glycocerebroside, an alkyl- β -D-glycopyranoside, or a phosphoglycolipid.

41. (New) The process according to claim 40, wherein the protein having the enzymatic activity of a processive lipid glycosyl transferase has an amino acid sequence which is more than 95% identical to the sequence selected from the sequences in the group consisting of SEQ ID NO. 2 and SEQ ID NO. 4.

E6
Cont

Appl. No. : 09/668,788
Filed : September 22, 2000

42. (New) The process according to claim 40, further comprising recovering the glycosyl diacylglycerol, the sterolglycoside, the glycocerebroside, the alkyl- β -D-glycopyranoside, or the phosphoglycolipid synthesized by the enzymatic activity of the processive lipid glycosyl transferase.

43. (New) The process according to claim 1, wherein the protein having the enzymatic activity of a processive diacylglycerol glycosyltransferase comprises an amino acid sequence selected from the group consisting of MITKPGGITXTE (SEQ ID NO. 8), VKXTGIP (SEQ ID NO. 9), ZPDIIIXXXP (SEQ ID NO. 10), more than 5 amino acids from within the sequence EHQPDI (SEQ ID NO. 5), more than 6 amino acids from within the sequence QVVVVC (SEQ ID NO. 6), and more than 6 amino acids from within the sequence DCMITKPG (SEQ ID NO. 7).

44. The process according to Claim 1, further comprising recovering the glycosyl diacylglycerol, the sterolglycoside, the glycocerebroside, the alkyl- β -D-glycopyranoside, or the phosphoglycolipid synthesized by the enzymatic activity of a processive diacylglycerol glycosyltransferase from the cell.
